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Authors

Corrubia, Lucas
Santhakumar, Viji

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Born to Be Wild: A Case for Targeting Ectopic Adult Born Granule Cells for Seizure Control

Altered Synaptic Drive Onto Birthdated Dentate Granule Cells in Experimental Temporal Lobe Epilepsy

Althaus AL, Moore SJ, Zhang H, Du Plummer X, Murphy GG, Parent JM. *J Neurosci.* 2019;39(38):7604-7614. doi:10.1523/JNEUROSCI.0654-18.2019.

Dysregulated adult hippocampal neurogenesis occurs in many temporal lobe epilepsy (TLE) models. Most dentate granule cells (DGCs) generated in response to an epileptic insult develop features that promote increased excitability, including ectopic location, persistent hilar basal dendrites (HBDs), and mossy fiber sprouting. However, some appear to integrate normally and even exhibit reduced excitability compared to other DGCs. To examine the relationship between DGC birthdate, morphology, and network integration in a model of TLE, we retrovirally birth-dated either early-born (postnatal day 7) or adult-born (postnatal day 60) DGCs. Male rats underwent pilocarpine-induced status epilepticus (SE) or sham treatment at postnatal day 56. Three to six months after SE or sham treatment, we used whole-cell patch clamp and fluorescence microscopy to record spontaneous excitatory and inhibitory currents from birth-dated DGCs. We found that both adult-born and early-born populations of DGCs recorded from epileptic rats received increased excitatory input compared with age-matched controls. Interestingly, when adult-born populations were separated into normally integrated (normotopic) and aberrant (ectopic or HBD containing) subpopulations, only the aberrant populations exhibited a relative increase in excitatory input (amplitude, frequency, and charge transfer). The ratio of excitatory to inhibitory input was most dramatically upregulated for ectopically localized DGCs. These data provide definitive physiological evidence that aberrant integration of post-SE, adult-born DGCs contributes to increased synaptic drive and supports the idea that ectopic DGCs serve as putative hub cells to promote seizures.

Significance Statement: Adult DGC neurogenesis is altered in rodent models of TLE. Some of the new neurons show abnormal morphology and integration, but whether adult-generated DGCs contribute to the development of epilepsy is controversial. We examined the synaptic inputs of age-defined populations of DGCs using electrophysiological recordings and fluorescent retroviral reporter birth-dating. Dentate granule cells generated neonatally were compared with those generated in adulthood, and adult-born neurons with normal versus aberrant morphology or integration were examined. We found that adult-born, ectopically located DGCs exhibit the most pro-excitatory physiological changes, implicating this population in seizure generation or progression.

Targeting Seizure-Induced Neurogenesis in a Clinically Relevant Time Period Leads to Transient But Not Persistent Seizure Reduction

Varma P, Brulet R, Zhang L, Hsieh J. *J Neurosci.* 2019;39(35):7019-7028. doi:10.1523/JNEUROSCI.0920-19.2019.

Mesial temporal lobe epilepsy (mTLE), the most common form of medically refractory epilepsy in adults, is usually associated with hippocampal pathophysiology. Using rodent models of mTLE, many studies including work from our laboratory have shown that new neurons born around the onset of severe acute seizures known as status epilepticus (SE) are crucial for the process of epileptogenesis and targeting seizure-induced neurogenesis either genetically or pharmacologically can impact the frequency of chronic seizures. However, these studies are limited in their clinical relevance as none of them determines the potential of blocking new neurons generated after the epileptogenic insult to alleviate the development of chronic seizures. Therefore, using a pilocarpine-induced SE model of mTLE in mice of either sex, we show that >4 weeks of continuous and concurrent ablation of seizure-induced neurogenesis after SE can reduce the formation of spontaneous recurrent seizures by 65%. We also found that blocking post-SE neurogenesis does not lead to long-term seizure reduction as the effect was observed only transiently for 10 days with >4 weeks of continuous and concurrent ablation of seizure-induced neurogenesis. Thus, these findings provide evidence that seizure-induced neurogenesis when adequately reduced in a clinically relevant time

period has the potential to transiently suppress recurrent seizures, but additional mechanisms need to be targeted to permanently prevent epilepsy development.

Significance Statement: Consistent with morphological and electrophysiological studies suggesting aberrant adult-generated neurons contribute to epilepsy development, ablation of seizure-induced new neurons at the time of the initial insult reduces the frequency of recurrent seizures. In this study, we show that continuous targeting of post-insult new neurons in a therapeutically relevant time period reduces chronic seizures; however, this effect does not persist suggesting possible additional mechanisms.

Commentary

The hippocampal dentate gyrus is uniquely endowed with constitutive birth of new neurons, termed "neurogenesis," well into adult life. Despite recent data questioning the extent of adult neurogenesis in humans, it is well established that neurogenesis in the subgranular zone of the dentate gyrus gives rise to new cohorts of adult born granule cells (abGCs), the principal cells of the dentate.[1,2] Addition of abGCs to the circuit occurs through a highly regulated process and is proposed to mediate indexing of memory traces and spatial context processing functions integral to the dentate.[3] Consistent with their proposed role in memory, exercise and enriched environment enhance neurogenesis and improve memory performance.[4] However, studies in diseases including temporal lobe epilepsy have recognized that increases in neurogenesis triggered by brain insults are not always benign. Indeed, while augmenting neurogenesis may compensate for neuronal loss, newly born neurons which develop in an altered circuit could augment excitability and promote epileptogenesis.[5-7] Prior work has identified that abGCs generated after experimental status epilepticus (SE) can migrate ectopically and show dendritic alterations including persistent basal dendrites suggesting that they may contribute to network dysfunction. Consistently, depletion of neurogenesis before SE can reduce subsequent occurrence of recurrent seizures, suggesting that abGCs may be targeted to limit epilepsy.[8] However, in light of abGC contributions to normal circuit function, blanket suppression of neurogenesis may not be ideal. In order to effectively manipulate neurogenesis for therapy, it is imperative to know whether the entire pool of SE-induced abGCs or merely a subset of "rogue abGCs" engage in abnormal activity and to determine when and for how long neurogenesis needs to be suppressed to limit epileptogenesis. The 2 studies summarized below use complementary approaches to address these important questions. By viral labeling of granule cells born before and after experimental SE or saline treatment, Althaus et al (2019) examined synaptic inputs to distinct cohorts of abGCs in hippocampal slices to test whether structurally distinct populations of SE-induced abGCs differ in network integration. Using genetic strategies to transiently ablate neurogenesis in vivo, Varma et al (2019) evaluated the effect of suppressing neurogenesis on development of recurrent spontaneous seizures. Together the studies aimed to address gaps in knowledge regarding what and when to manipulate SE-induced neurogenesis to reduce risk of acquired epilepsies.

The study by Althaus and colleagues delved into abGCs generated early after pilocarpine-SE in rats and examined their synaptic inputs in a presumed epileptic network after 3 to 6 months. The remarkable temporal specificity in labeling early-born granule cells (ebGCs), born a week after birth and prior to SE, versus abGCs born 4 days after SE-induction/saline-treatment, was achieved by transfecting dentate neural precursors with an engineered retrovirus expressing a fluorescent reporter. Injection of the retrovirus, which selectively infects dividing cells for about a 6-hour period, allowed for selectively tagging ebGCs or abGCs generated after SE. They identified that the somata of all ebGCs, regardless of seizures, and abGCs in control rats remained restricted to the granule cell layer. Although a large population of post-SE abGCs showed normal structure and location, subsets of SE-induced abGCs showed abnormal basal dendrites or were located "ectopically" outside the cell layer. Analysis of the spontaneous synaptic inputs to the birth-dated neurons identified that ebGCs receive slightly more and larger amplitude glutamatergic inputs and fewer GABAergic inputs. Together, these changes shift the "charge transfer," or the net effect, of basal synaptic inputs in post-SE ebGCs toward greater basal excitatory drive. Interestingly, among abGCs, only those with abnormal location or structure showed increases in steady-state excitatory drive after SE. Ectopic abGCs showed the most robust increase in both amplitude and frequency of excitatory drive than abGCs in the cell layer or with basal dendrites. Inhibitory synaptic inputs to SE-induced abGCs were largely unchanged, contrasting with the decrease observed in ebGCs after SE. Overall, "abnormal" abGCs with ectopic location and basal dendrites showed a striking enhancement of basal excitatory charge transfer indicating that they are likely recruited more readily during network activity. The excitation/inhibition (E/I) ratio of charge transfer presents an intuitive synthesis of the net effects of the multiple changes in excitatory and inhibitory synaptic parameters. However, as noted by the authors, the use of average data drawn from spontaneous excitatory events recorded in GABA receptor antagonists and spontaneous inhibitory events isolated in glutamate receptor blockers from a different group of cells to calculate the E/I ratio suggests that it may not represent conditions in the intact network. Regardless, the study identifies that ectopic abGCs receive greater excitatory drive and complement prior findings that ectopic abGCs are more excitable.[9] These results open the potential for identifying and targeting the molecular cues that drive the abnormal

localization and structure of post-SE abGCs to limit epileptogenesis. The intriguing results that ebGCs and not abGCs receive reduced inhibitory drive after SE, while increases in glutamatergic drive are an order of magnitude higher in ectopic abGCs indicate that ebGCs and ectopic abGCs integrate very differently into the circuit and begs the question of whether they target different postsynaptic neurons.

Varma and colleagues build on their prior demonstration that transient suppression of neurogenesis before pilocarpine-SE in mice reduces subsequent development of recurrent spontaneous seizures.[10] Using transgenic (Nestin-herpes simplex virus thymidine kinase or Nestin-TK) mice in which administration of ganciclovir transiently and reversibly ablates neurogenesis, they find that ablating neurogenesis for 4 weeks immediately following SE was unable to suppress seizure frequency or duration when examined 5 to 7 weeks after SE. The lack of effect on spontaneous seizures occurred in spite of a dramatic reduction in newborn neurons labeled with doublecortin or hilar ectopic abGCs, compared to post-SE mice receiving vehicle treatment. Mice examined for seizures 5 to 7 weeks after SE, during continued ablation of neurogenesis for 8 weeks, showed reduction in the frequency of spontaneous recurrent seizures, with no change in seizure duration. Neurogenesis and hilar ectopic abGCs remained suppressed 10 weeks after the end of 8-week ganciclovir treatment yet failed to reduce seizure frequency. These findings demonstrate that although suppression of neurogenesis *after* SE can limit seizures, both early cohorts of post-SE abGCs and those generated subsequently may contribute to seizure recurrence. Alternatively, prolonged suppression of neurogenesis may enhance other mechanisms leading to epilepsy. The data differ from the prolonged reduction in seizure frequency when neurogenesis is depleted prior to SE or with near-complete and permanent elimination of neurogenesis after SE.[7,10] Curiously, although recovery of neurogenesis following ablation was relatively low, the data suggest that transient ablation may be ineffective in the long run. In this regard, inclusion of non-SE controls to assess time line and efficacy of recovery of neurogenesis would have helped evaluate whether the surprising lack of recovery in neurogenesis for over 10 weeks after end of the transient ablation was unique to the disease process and identify whether even limited and delayed post-SE neurogenesis could promote seizures. Additionally, since recent work indicates a role of abGCs in supporting feedback inhibition,[11] it would be interesting to consider whether prolonged, nonselective prolonged suppression of neurogenesis which reduces functionally normal abGCs in the cell layer, in addition to the hilar ectopic abGCs, may compromise feedback inhibition and promote epileptogenesis. Overall, Varma et al (2019) demonstrate that although transient suppression of neurogenesis after SE may delay progressive increase in seizure frequency, the effects do not persist long term. Further studies are necessary to assess whether "abnormal" abGCs and cell-layer abGCs have differential effects on epileptogenesis and whether treatments resulting in prolonged suppression of neurogenesis after SE adversely impact dentate function.

The 2 studies conducted in different rodent models using distinct approaches, together, provide a more detailed picture of the consequences of post-SE neurogenesis in space and time. Although Althaus et al (2019) identify that a subset of SE-induced abGCs receive enhanced excitatory drive, suggesting that they may have gone rogue, Varma et al (2019) support a role for neurogenesis, acting in concert with other circuit mechanisms, in promoting recurrent seizures. Further studies are needed to determine the cues that prompt a subset of abGCs, generated after seizures to migrate ectopically and receive enhanced glutamatergic drive. Although the role of ectopic abGCs in augmenting network excitability remains to be determined, the ablation paradigm adopted by Varma et al (2019) was unable to prevent seizures despite reducing ectopic abGCs, raising the possibility that ectopic abGCs may not be the primary drivers of epileptogenesis. Alternatively, since the ablation protocol reduced abGCs with presumably normal location and synaptic inputs, it is possible that benefits of reducing "ectopic" abGCs are offset by circuit changes resulting from other pathological processes. Thus, while suppressing neurogenesis after SE may be partially effective in delaying disease progression, it appears insufficient to prevent epileptogenesis and may need to be combined with other mechanistically based strategies to achieve seizure prevention.

By Lucas Corrubia and Viji Santhakumar

ORCID iD

Viji Santhakumar https://orcid.org/0000-0001-6278-4187

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